Current Biology 19, R772–R780, September 15, 2009 ©2009 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2009.07.063

Domain-Driven Morphogenesis of Cellular Membranes

Review

Anna V. Shnyrova, Vadim A. Frolov, and Joshua Zimmerberg*

Cellular membrane systems delimit and organize the intracellular space. Most of the morphological rearrangements in cells involve the coordinated remodeling of the lipid bilayer, the core of the membranes. This process is generally thought to be initiated and coordinated by specialized protein machineries. Nevertheless, it has become increasingly evident that the most essential part of the geometric information and energy required for membrane remodeling is supplied via the cooperative and synergistic action of proteins and lipids, as cellular shapes are constructed using the intrinsic dynamics, plasticity and self-organizing capabilities provided by the lipid bilayer. Here, we analyze the essential role of proteo-lipid membrane domains in conducting and coordinating morphological remodeling in cells.

Introduction

The cell as a whole, as well as its many intracellular organelles, should remain mechanically coherent, capable of cooperative restructuring and/or force transduction [1]. This 'whole-cell' coherence is supported by sophisticated filamentous networks responsible for long-range ordering of cellular cytoplasm and cortex [1], but it does not directly apply to the membrane system that defines the physical barriers delimiting the cell and its compartments. Membranes retain substantial conformational plasticity at subcellular, submicron scales, at which they are constantly reshaped as they form transport vesicles, membrane protrusions and invaginations of different shapes [2,3]. These shape changes are conducted by specialized proteins (such as coatomers [4], which coat membrane transport vesicles) that self-assemble on the lipid membrane into local modules [3,5,6]. The coordination of the activity of these modules is essential for supporting and controlling membrane shapes at larger scales [7,8]. Consequently, the 'whole-cell' spatial ordering of membrane formations by filaments also relies on distinct, spatiotemporally arranged membrane modules [9,10].

The view of the cellular membrane as a multifaceted and heterogeneous entity [11,12] is in good agreement with this apparent modular design of membrane reorganization. Indeed, small submicron membrane domains of distinct composition can be readily perceived as precursors of transport vesicles [13–15], transient membrane protrusions [16,17], sites of membrane fusion and fission [18] and invaginations of plasma membrane, like caveolae [19,20] (Figure 1A). These domains are enriched with proteins that are specialized in membrane remodeling and often associated with particular lipid species implicated in domain formation, such as multivalent charged lipids, ceramides or cholesterol [6,18,20–22].

How does the segregation of protein and lipid components yield distinct, yet dynamic patterns of membrane shape? The answer to this important question becomes increasingly complex. The initial paradigm was invoked by ultrastructural techniques that revealed reorganization of the submembrane actin cortex and formation of membrane-associated protein meshes (primarily cages formed by coat proteins) in places of membrane deformation [23–25]. These polymerized protein structures were initially considered to be three-dimensional scaffolds imposing their geometric wishes on the lipid bilayer [1,26,27]. However, besides coats and filaments, membrane remodeling requires additional players, such as adaptor proteins in coated pits and actin–membrane linkers [3,28–31]. These proteins substantially alter the membrane microstructure, creating nucleation spots for membrane remodeling [9,13]. Importantly, these proteins can directly change membrane curvature through specialized and often evolutionarily conserved protein domains (e.g. ENTH or BAR domains [32,33]), which generally recognize specific lipid species [18]. Thus, the 'scaffold' paradigm also has to include these accessory proteins and lipids as complementary factors that mediate the initial localized membrane deformations, which are then ultimately coordinated in space by the scaffold. This task distribution corresponds to the layered (or modular) structure of the proteo-lipid machinery that shapes membranes: the inner layer directly embedded in the lipid bilayer contributes substantially to the work of bending, while the outer protein scaffold that lies over the bilayer brings in the geometric information [5,9,34].

Nevertheless, recent experimental data indicate that this proposed hierarchy is not strictly applied. In fact, protein scaffolds might be very dynamic structures that adopt different shapes [25,35–38]. In addition, some curvature-driven proteins can guide membrane deformations without a scaffold, in both *in vivo* and *in vitro* systems [29,30,39,40]. Instead of rigid structures, these proteins can assemble dynamic membrane domains, the shape of which is controlled by elastic and boundary forces, similar to fluid-like lipid domains [41,42]. This analogy is further corroborated by the sensitivity of membrane remodeling in cells to domain-forming lipids [20,43,44] and, in part, by the general involvement of lipid domains in intracellular membrane dynamics [45–48]. The forces lying behind the assembly of such membrane domains can be fully responsible for local membrane deformation, as experiments on purely lipid systems have demonstrated [49,50] (Figure 1B). Thus, the proteo-lipid machinery governing localized membrane remodeling in cells involves elements of dynamic self-organization, brought by the lipid bilayer [18,43]. Here, we review recent experimental and theoretical data supporting the emerging role of integrated proteo-lipid modules in cellular morphogenesis.

In order to better illustrate this modular principle, we introduce the concept of the morphological domain (MOD), which

Laboratory of Cellular and Molecular Biology, Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892-1855, USA.
*E-mail: zimmerbj@mail.nih.gov

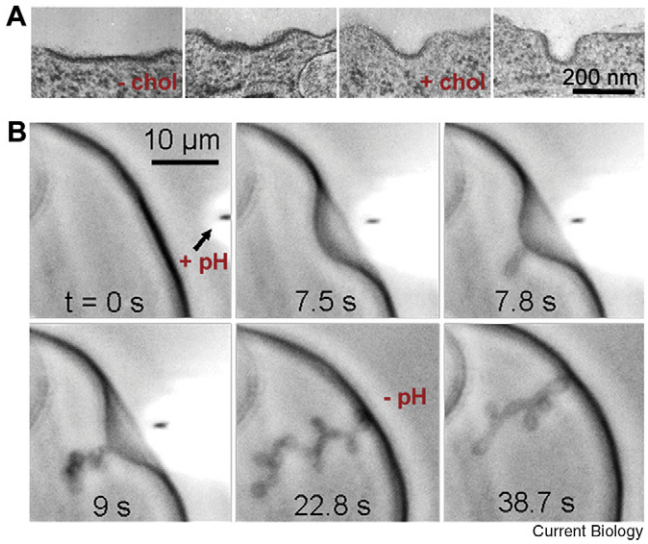

Figure 1. Membrane deformations triggered by changes in membrane composition.

(A) A caveola adopts its characteristic invaginated shape in the presence of cholesterol (adapted from [48]). (B) Deformation of giant lipid vesicles induced by localized protonation of cardiolipin (from [49]).

we define as a dynamic proteo-lipid module undergoing morphological maturation through self-organization. The MOD is not regarded as a 'classical' raft [45], a concept primarily based on self-assembly of lipid domains that arise through separation of lipid phases to which proteins partition. Indeed, there are many examples of biological membrane domains that are composed of lipids that mix well with their neighbors. Thus, we would like to expand our description of cellular membrane domains beyond the classical raft concept. Rather, the MOD incorporates dynamic links between the composition and the shape of complex proteo-lipid membranes. These links are vital for the functional coupling of cellular membrane transport and signaling networks [51–55], cell motility [17,54,56] and *de novo* creation of intracellular organelles [14,57].

MOD Concept

The coupling between the shape and composition of MOD is directly related to the energies and spatial organization of membrane deformations. The lipid bilayer is relatively flaccid at the submicron scales characteristic for MODs. For example, the hydrolysis of about 50 ATP molecules provides approximately 500 k_BT, the energy sufficient to form a spherical lipid vesicle [3,42]. For coated vesicles, this energy can be supplied by ATPases, such as Hsc70 [4,58]. Such energy estimates, however, are generally based on rigidities that are typical for model phospholipid membranes. Cellular membrane patches tightly packed with proteins might be substantially stiffer [3]. Lipid bilayers at higher curvatures are also less bendable: formation of highly curved membrane intermediates, especially those involved in fusion and fission processes, is very demanding energetically. The analysis of these intermediates underlines the major role of membrane composition in controlling the pathways of membrane remodeling [59,60]. Lipid composition and geometry should be perfectly coordinated in order to minimize the energetic cost of membrane deformations, particularly those associated with the creation of high membrane curvature [44,59,61].

Even if the energy for membrane deformation were available, it still needs to be converted into a cooperative restructuring of a large molecular ensemble — the membrane patch containing many protein and lipid molecules (e.g. 100-nm vesicles contain $\sim 10^5$ lipids). The transduction of energy to the membrane is generally achieved via specialized adaptor/linker proteins, which contain specific functional domains that directly interfere with the structure of the lipid bilayer [29,32,33]. Many such adaptors, e.g. epsin [30], themselves induce changes in membrane geometry. However, a substantial membrane fraction of these proteins is required in order to change membrane shape [62], as they likely need to be segregated to amplify and coordinate their curvature activity [63]. By enforcing the segregation of these adaptor proteins and/or applying specialized enzymes that produce non-bilayer lipids [59], cells actively adjust composition to form curved membrane domains. In principle, such domains can be either of crystalline nature (e.g. protein caps [64,65]) or weakly bound fluid-like formations that can still ensure the local coherence of membrane shape dynamics [42,45]. The role of these fluid-like domains in membrane morphogenesis is only beginning to be appreciated [20,42,66]. Such dynamic formations may be at the core of the energy transduction and spatial organization of membrane remodeling at submicron scales.

The notion of membrane domains as dynamic clusters of components already plays an important role in our understanding of cellular membrane organization [11,46]. Compositionally defined spontaneous segregation of lipids into fluid-like domains was instrumental in the development of the concept of dynamic heterogeneity of cellular membranes [45,46]. Furthermore, changes of membrane geometry in turn can trigger lateral redistribution of lipids [67] and attract curvature-sensing proteins [3,6]. Importantly, the curvature-induced sorting of lipids is manifested either at high ($> 0.1\ nm^{-1}$) curvatures or when the lipid mixture is close to the point of spontaneous decomposition into domains [67–69]. Thus, the two processes, the composition-driven and the curvature-driven segregation of membrane components, are intimately linked and this link provides a basis for the appearance of MODs. The stationary shapes of MODs can be described in traditional terms of the intrinsic curvature characteristic for each particular membrane composition.

Intrinsic Curvature and MOD Shape

The intrinsic curvature of a homogeneous lipid monolayer containing a single lipid component is dictated by the minimization of the elastic stresses (i.e. stretching and squeezing) imposed on lipid heads and tails during the self-assembly of the monolayer [3]. The monolayer bends to minimize these stresses, thus adopting its intrinsic shape. As a result, the lipid molecules acquire certain average geometry [70,71]. For a multi-component lipid monolayer this geometry might be a complicated function of its composition [72], making predictions of membrane intrinsic curvature difficult. However, the direction of monolayer bending can be evident when a dominating curvature-active agent is present. For example, the so-called non-bilayer lipids, characterized by extremely high values of intrinsic curvature, impose positive (e.g. lyso-lipids [72]) or negative (e.g. diacylglycerol [73]) curvatures (shown as red and blue triangles, respectively, in Figure 2A).

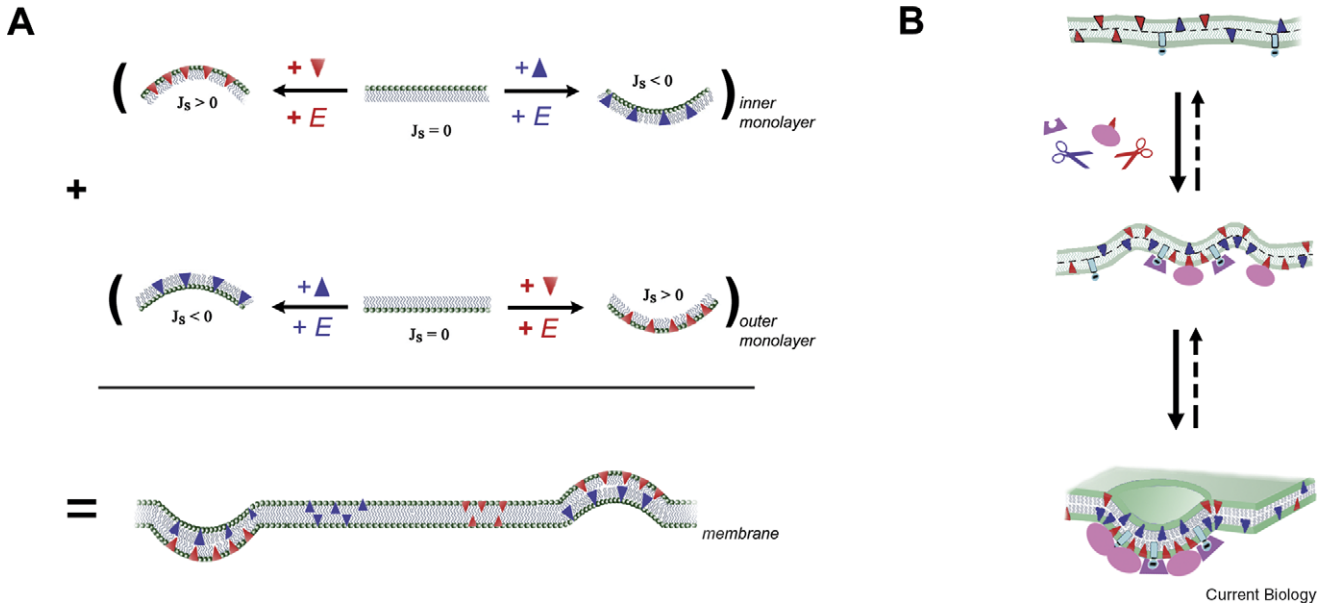

Figure 2. Intrinsic curvature and emergence of MODs.

(A) Addition of non-bilayer lipids changes the intrinsic shape of the lipid monolayer producing positive (lyso-lipids, red triangles) or negative (diacylglycerol, blue triangles) curvature; the intrinsic curvature of the lipid bilayer is a combination of those of its monolayers. Monolayers of the same intrinsic curvature and stiffness can make a flat bilayer. (B) Appearance of MODs as a result of curvature instabilities. Creation of curved areas in the membrane, here due to thermal undulations or local enzymatic production of non-bilayer lipids (such as diacylglycerol, blue triangles and scissors; and lyso-lipids, red triangles and scissors), is coupled to the lateral redistribution of the membrane components according to their curvature preferences and to the curvature-driven binding of proteins. This coupling first leads to the enhancement of undulations, followed by the emergence of MODs when a critical amount of curvature-inducing components (pink) is attracted to the MODs, probably via specialized domains recognizing charged lipid species (blue caps). MODs can quickly disappear if the curvature-active components leave, e.g. via impairment of electrostatic interaction (e.g. removal of PIP_2 [53]).

The intrinsic state of the lipid bilayer is determined by the combined minimization of the elastic stresses in both of its monolayers. Some simple qualitative predictions of this minimization are depicted in Figure 2A. Similar 'molecular geometry' formalism can be used to predict the curvature activity of an individual protein interacting with the bilayer: a shallow hydrophobic insertion (e.g. the ENTH domain [30]) induces positive curvature in a monolayer, similar to a lysolipid molecule [72]. The protein insertion can be energy- and charge-dependent, thus providing means of quick and dynamic regulation of the intrinsic curvature of MODs [30,74].

Besides insertion domains, proteins that are specialized in controlling membrane curvature have developed curved domains that mold the lipid bilayer via electrostatic attraction of charged membrane species (e.g. the BAR domain [29] or the Sec23/24 complex [75]; reviewed in [2,3,6]). Finally, localized and synchronized insertion of numerous proteins can generate an area difference between the monolayers of the MOD membrane, leading to bending due to the coupling that exists between the two layers [3,76]. This area difference can be also achieved through the translocation of lipids from one monolayer to another by the action of specialized proteins (e.g. flippases [61]).

Creation of membrane curvatures characteristic of submicron MODs requires substantial membrane coverage by proteins working through changes in intrinsic curvature [62], resulting in protein crowding. This crowding by itself can drive changes in membrane shape [77]. Also, when proteins pack tightly in the MOD area, their packing preferences will impose particular shapes on the MOD. For example, the most dense surface coverage by curved and elongated BAR domains is achieved on a cylinder: BAR domains can completely cover a cylinder, but not a sphere [39,78]. Hence, BAR domains would readily accumulate on and stabilize cylindrical shapes even without polymerization in a multimolecular scaffold. Formation of cylindrical membranes with both positive (N- and F-BAR domains [79]) and negative (I-BAR domains [80]) curvatures has been reported in reconstituted systems and recently also in cellular systems in which I-BAR domains created dynamic membrane protrusions [81]. Thus, the protein-packing preferences, even without direct interaction between the proteins, can guide the MOD shape.

Interestingly, similar packing preferences can affect the polymerization of proteins interacting with MOD (Figure 3). Actin polymerization patterns can be altered by membrane curvature [28,42,82,83], so the formation of the supporting actin mesh inside the filopodia (Figure 3) can follow the creation of membrane protrusion by I-BAR-domain-containing proteins [81]. For classical protein coats, adsorption on a curved membrane surface can bias the formation of one of the multiple fullerene lattices assembled by these proteins in solution [24,84].

Certain proteins, caveolin in particular, extend their action on both membrane monolayers, summing up the differential insertion effects (induction of positive curvature in the contacting and negative curvature in the distant monolayer) with segregation of cholesterol [85,86]. This combination results in stationary membrane invaginations, known as caveolae, the stability of which relies on the cholesterol content in the membrane [48] (Figure 1B). Less stable MODs allow for detectable exchange of their protein components

with the bulk phase. These dynamics become extremely important at early stages of MOD development.

Emergence of MODs

The remodeling of a homogeneous membrane is initially restricted by an energy barrier related to the stability of the reference membrane shape. For example, forming a cylindrical membrane tether from a phospholipid bilayer is characterized by an initial jump in pulling force [87]. Self-assembly of a curved MOD can reduce this initial barrier, making the initial membrane deformation(s) spontaneous. The coupling between dynamic changes of membrane curvature on one hand and segregation and energy consumption by proteins embedded in the lipid bilayer on the other is summarized by the concept of 'active' membranes [88]. This concept reveals the leading role of the lipid bilayer and its intrinsic dynamics in the organization of non-equilibrium membrane deformations [16,88–90]. According to this idea, the emergence of MODs relies on a self-enhanced process that proceeds through the accumulation of curvature-driven components in curved membrane regions (Figure 2B). In an initially homogeneous membrane, this accumulation is coupled to the membrane undulations that cause initial deviations of membrane curvature [31,56,86]. To further enforce this positive feedback, curvature-driven components can be 'active', i.e. providing not only additional curvature, but also energy [56,91]. The outcomes of these positive feedback loops have been modeled for several cellular membrane systems. In the initiation of filopodia growth, accumulation of actin–membrane linkers leads to a concentrated pushing force of actin filaments into peaks of membrane undulations, leading to the further development of local membrane curvature and global curvature instabilities [16,56]. Similar positive feedback related to the creation of membrane curvature that is further sensed and enhanced by specialized proteins was revealed in modeling of formation of coat protein I (COPI)-coated vesicles in endoplasmic reticulum (ER) exit sites [92]. Finally, initiation of membrane deformations can be triggered through lipid modification by enzymes producing curvature-active membrane components (e.g. see [47]). Thus, dynamic curvature instabilities can lead to the appearance of MODs through positive feedback, including Turing or wave instabilities [16,93].

One of the expected manifestations of these instabilities in cellular systems is the stochastic behavior of dynamical cellular MODs. Fluorescent labeling of the individual components of MODs, allowing for time-lapse monitoring of MOD appearance, shows that the emergence of a clathrin-coated vesicle on the plasma membrane involves a stochastic process. The vesicle precursors (i.e. small assemblies of the vesicle proteins) constantly appear and disappear until one of them reaches a point of dynamic instability (or 'control' point) and progresses further into a mature vesicle [13,94,95]. Thus, the appearance of a MOD is unlikely to be template-driven, but rather a complex non-equilibrium process, stochastically directed towards shapes dictated by the curvature preferences of specialized proteins.

Maturation of MODs

In general, the appearance of MODs triggers a response from the downstream cellular systems that support the further development of these domains [96]. This triggering can be induced even by endogenous molecules that form curved domains within the plasma membrane and undergo endocytosis [97]. This reaction is supported by curvature sensing, the characteristic ascribed to protein domains [3] and, recently, to polymerization patterns of actin [81,83]. In more tightly regulated membrane remodeling events, such as receptor-mediated endocytosis, the appearance of the initial MOD triggers the hierarchical recruitment of various proteins involved in curvature creation, cargo selection and overall maturation of endocytic pits [13,98]. In this way, the maturation of the MOD switches from dynamic self-organization of a proteo-lipid domain, characteristic at the stage of MOD emergence, to virtually irreversible, template-driven development.

Physically, the dynamic proteo-lipid MOD becomes associated with a layer (or layers) of curvature-stabilizing proteins (scaffolds and supporting filaments), which ensure the unidirectionality of MOD shape development and stabilize intermediates in this process. The recent experiments, however, question whether this second layer of morphological control is a stable shape template or whether the MOD remains dynamic, supporting the original self-organizing character of MOD maturation (this dilemma is depicted in Figure 4A, left and right pathways, respectively).

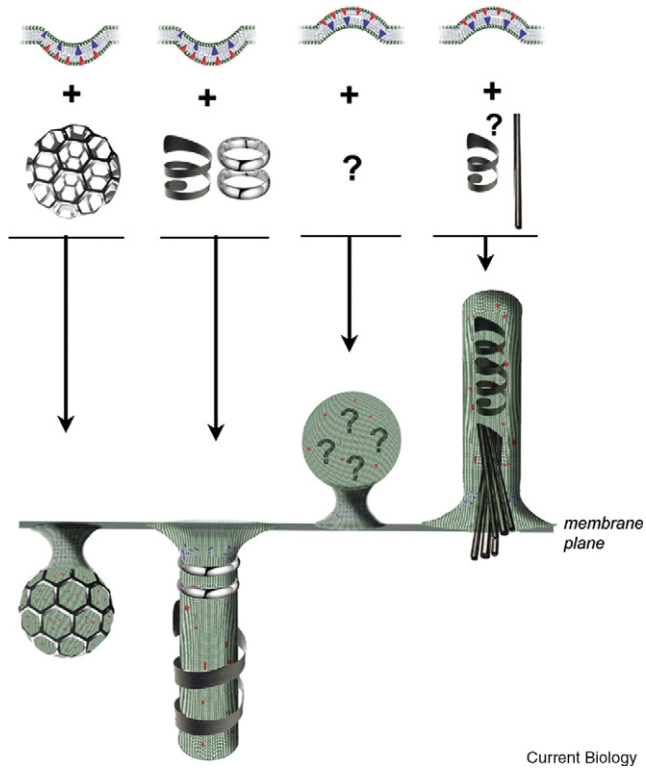

Figure 3. Proteins shaping MODs.

The right part of the figure shows the creation of MODs in the outward direction away from the cytoplasm. Actin and I-BAR domains assemble inside the MOD according to their polymerization pattern (actin) or packing preferences (I-BAR domains), transforming membrane invagination into a cylindrical filopodia. Proteins, like viral matrix proteins producing virus-like particles [120] or CHIMP/ESCRT complexes [121], can transform membrane invagination into a spherical bud, but the mechanisms that ensure spherical topology remain largely unknown. The left part of the figure shows the formation of cylindrical and spherical MODs directed towards the cytoplasm by proteins forming rings, spirals (BAR domains, dynamin) and spherical cages (clathrin). Straight filaments (actin) are likely to support the cylindrical phenotype and apply axial forces (red arrows) perpendicular to the parent membrane.

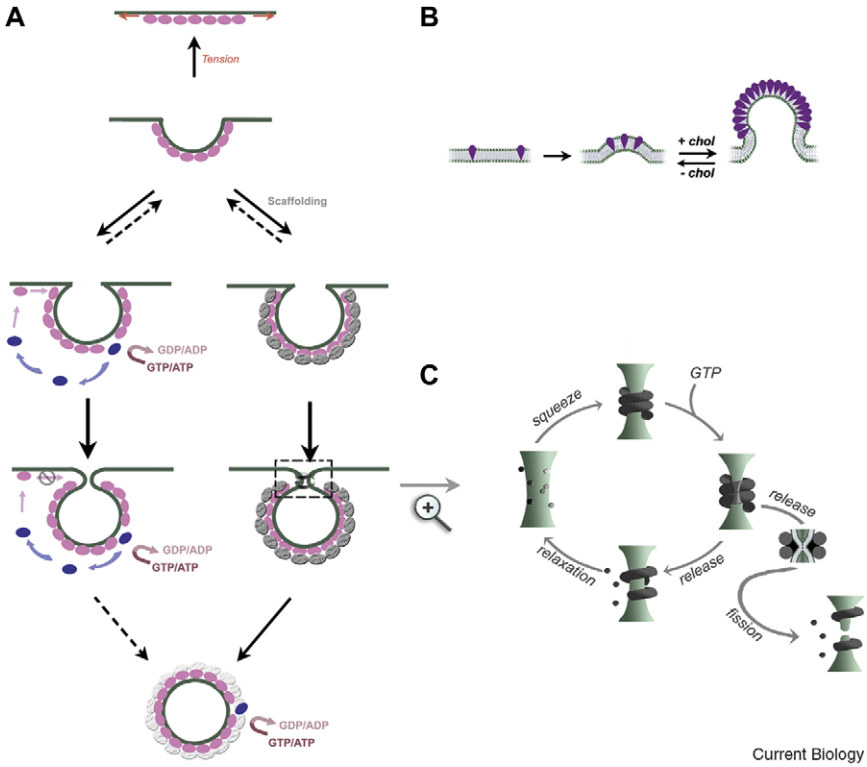

Figure 4. Maturation of MODs.

(A) The two alternative pathways of spherical MOD development. The left branch illustrates the dynamic coat hypothesis where the coatomers are in constant, energy-dependent exchange with the bulk phase (permanent uncoating [4]). Their concentration in the MOD is defined by the balance between influx through the MOD neck (pink) and efflux through detachment from the MOD (blue). The influx, stimulated by MOD curvature (positive feedback), dominates, supporting the stable coverage of MOD membrane by the coatomers. Narrowing of the MOD neck cuts off the influx (negative feedback), ensuring that MOD uncoating precedes vesicle detachment [108]. The right branch illustrates the polymerization of a stable coat triggered by emergence of an initial membrane invagination. Formation of this invagination can be impaired by lateral membrane tension (red arrows), which tends to flatten the MOD. Evolution of the spherical MOD inevitably leads to the appearance of a new MOD, the neck (square). For fluid-like domains, the neck formation is promoted by the line tension (black arrows). (B) Cholesterol-dependent formation of caveolae. (C) Dynamic changes of the neck shape: the squeeze and release cycle produced by reversible assembly of dynamin spirals on the neck membrane coupled to cooperative GTP hydrolysis by the protein is illustrated. These cycles can produce fission of the neck as a stochastic outcome [116,122].

Self-Organization and Stabilization of MOD Shape

The attractive interactions that stabilize MODs can be mediated by lipid components, cholesterol in particular, which are involved in the formation of fluid-like lipid membrane domains [45]. Similar cholesterol-dependent stabilization has been reported for MODs formed by caveolin and flotillins (Figure 4B) [20,99]. This composition-driven attraction can be supplemented by protein recognition ('sensing') of the MOD curvature and charge, mediated by specialized protein domains [6,100]. Highly charged lipids, primarily inositols, are deeply involved in the regulation of membrane trafficking and morphology in cells [53], making membrane electrostatics a powerful tool in regulating membrane dynamics. Finally, MODs can be stabilized by forces related to protein insertion into the lipid bilayer. It has been estimated that the interference of membrane deformations produced by proteins embedded into the lipid bilayer might lead to weak attractions between neighbor proteins, stimulating the protein clustering [63,101,102]. However, direct experimental confirmation of the involvement of these attractive forces acting on proteins during membrane remodeling is still lacking. The curvature of MODs not only affects the proteins, but also the lipids. It has been experimentally demonstrated that changes of membrane curvature can also induce segregation of lipids [67]. Thus, MOD curvature is intimately involved in controlling and maintaining MOD composition.

The attractive forces related to the lipid matrix of MODs generally induce two types of ordering within MODs — fluid-like and crystalline — both of which have been explored in pure lipid systems [45,103]. The crystalline ordering, as an external proteinaceous scaffold, provides a stable template for MOD shape. Recent experimental data, however, provide the following arguments against membrane shape creation by a stable template.

The first set of arguments is related to the conformational plasticity of classical shape templates on the membrane surface, e.g. clathrin assembles into large flat sheets, tubes and spheres [35,104]. The assembly, and thus the resulting shapes, of the clathrin mesh depend on interactions with the MOD components [105]. The shape of a clathrin lattice is dramatically altered upon depletion of one of the endocytic accessory proteins, CALM [36]. Formation of large holes in the lattice does not compromise membrane curvature creation [36], indicating that the integrity of the protein lattice is not a requirement for membrane remodeling.

The second set of arguments comes from real-time observations of the dynamics of individual components of different cellular MODs. Recent experimental data indicate that the components of the coat protein scaffold *in vivo* remain dynamic and exchange with the bulk phase in an energy-dependent manner. This exchange is evident in the perpetual treadmilling of protein filaments involved in cell motility [56,106,107]. Also, the components of clathrin and COP coats can exchange with the cytoplasmic bulk phase during the maturation of their MODs [31,38]. This recycling depends on membrane curvature and the presence of small GTPases (e.g. Arf1) and ATPases (e.g. Hsc70) that are known to be involved in the regulation of membrane binding and the self-assembly of coat proteins [13,58,74,94,95,108]. The involvement of GTPases and ATPases in this process indicates that MOD maturation requires energy [26]. Interestingly, this recycling might be retained through the whole maturation of MODs, providing

negative feedback between MOD composition and shape (Figure 4A) [108,109]. In this way, the supply of MOD components is cut off automatically upon completion of shape creation, thus demonstrating a feature of MOD maturation: self-regulation [109]. Similarly, MOD maturation can be altered by cargo proteins, which, following accumulation on the MOD membrane, hinder the exchange of MOD components [110]. Finally, it is important to emphasize that some MODs have both rigid-template and dynamic phenotypes depending on the lipid components. An example is provided by caveolae, whose stable appearance depends dramatically upon the presence of cholesterol (Figure 4B).

All of these dynamic, non-equilibrium aspects of scaffolding cannot be deduced from static electron microscopy images. Thus, the unequivocal interpretation of the role of the coats and filamentous structures seen in places of membrane deformations is still ahead of us. Perhaps, what has become clearer is the universal involvement of the curvature-driven agents in the initiation and development of MODs. So, at least for some membrane systems, MOD maturation can be considered in the context of a dynamic interaction between a self-organized, fluid-like membrane domain and a protein template with a role in stabilizing the domain shapes. Interestingly, the proposals of dynamic maturation and the template model are not mutually exclusive. It has been proposed that stabilization of the MOD shape might happen in a step-wise manner [31]. According to the Brownian ratcheting principle, a MOD's growth is driven by random thermal fluctuations in its shape, and the template catches and stabilizes only those shapes that match the template geometry.

Forces Acting on MODs

Besides the forces related to the molecular interactions within a MOD, its shape also depends on the external forces acting on the MOD membrane. As membrane remodeling is often associated with reorganization of membrane cortex, the forces produced by actin filaments are generally implicated in membrane remodeling. Force-induced membrane deformations — pulling or pushing the tubular membrane protrusions by filament polymerization or molecular motors — have been reconstituted *in vitro* [90,111]. Motors are also involved in the creation of tubular carriers in the ER [112]. Formation of the tube requires cooperative action of motor proteins, illustrating the stiffness of the lipid bilayer [31]. Importantly, the stationary composition of the tubular extension can differ from that of the parent membrane [67]: the tubular MOD can optimize its composition:curvature ratio. This ratio apparently depends on the force which can therefore stabilize MODs of particular composition and geometry. The competition between the external force and the intrinsic wishes of the MOD, as dictated by its composition, should determine the shape dynamics of cylindrical MODs, such as filopodia [113].

Another force factor acting at the MOD boundary is associated with the lateral tension of the parent membrane. The effect of lateral tension depends crucially on the MOD geometry. For cylindrical MODs, tension acts as the pulling force applied along the cylinder axes, squeezing this MOD and promoting corresponding changes in its composition [114–116]. High lateral membrane tension can completely inhibit the emergence of the initial MOD [94,117] (Figure 4A). This effect of the lateral tension is important for understanding how multiple spherical MODs in the same membrane can be coordinated by a single force.

Finally, there is a force inevitably associated with the MOD edge. This force is intrinsically linked to the fact that the molecules localized on the edge want to be inside the MOD. Therefore, this force tends to diminish the domain edge [41,65]. For fluid-like membrane domains, this force is determined by line tension, which is the energy per unit of domain boundary length [41]. This edge energy can be minimized by decreasing the size of the edge through transformation of a planar or slightly curved domain with a large edge into a spherical bud with a small edge [41]. Thus, line tension stimulates maturation of MODs and generally imposes spherical morphology.

The Neck MOD

The termination of MOD shape maturation leads to the appearance of a special MOD — the neck MOD, a generic saddle-like intermediate in topological membrane remodeling, fusion and fission (Figure 4A,C). This MOD forms only in the context of membrane transport, so that its appearance is closely linked with the formation of other MODs. For example, the appearance of the neck MOD can finalize the maturation of a spherical MOD (Figure 4).

The neck MOD is usually associated with ring-like assemblies of proteins, such as the GTPase dynamin or fusion proteins [59], in boundary regions that generate membrane deformations involved in membrane remodeling in a small membrane area encircled by the rings. In this way, the action of the protein machinery assembled in this MOD is focused on creation of small and highly bent intermediates that are commonly involved in membrane fusion and fission. Subsequently, the saddle-like geometry of the neck MOD is essential to minimize the bending energy of highly curved membranes [118]. The sizes of protein assemblies on the neck's boundary are determined by the geometric preferences of the neck MOD.

Importantly, the neck MOD remains very dynamic, as revealed by estimates of its dimensions from electrophysiological measurements of the ionic conductivity through the internal lumen of the neck [115]. Reconstitution of dynamin-driven membrane neck remodeling on lipid nanotubes reveals that shape dynamics are coupled to the cyclic assembly and disassembly of short dynamin scaffolds fueled by GTP hydrolysis (Figure 4C). This observation further corroborates the view of the dynamic and stochastic nature of MODs in cellular membranes.

Concluding Remarks

Cellular membranes form a very crowded, heterogeneous environment characterized by extremely complex dynamics. Yet cells have developed various ways in which they keep their membrane systems under strict control, ranging from global transformations of cellular architecture to localized membrane remodeling. This dynamic ordering of cellular morphology is unimaginable unless membranes have the means to self-organize at small, subcellular scales. Cellular membranes self-organize through domains [11], each of which has a mission closely linked to its composition, e.g. clustering of channels or receptors, precursors of transport vesicles, or places of focal adhesion [51,92,119]. Membrane remodeling is initiated through self-organization of morphological domains characterized by particular shape preferences. Through their composition, these domains provide

a crucial link between intracellular energy and transport networks and membrane morphology. Membrane domains are the generic units of self-organization of cellular membrane shape.

Acknowledgments

We apologize that space limitations did not allow us to cover all of the newly emerging paradigms in the rapidly changing fields of cellular morphogenesis and membrane dynamics or include many of the important references to original experimental findings. Work in Zimmerberg's lab is supported by the Intramural Research Program of the NIH, NICHD.

References

1. Cai, Y., and Sheetz, M.P. (2009). Force propagation across cells: mechanical coherence of dynamic cytoskeletons. Curr. Opin. Cell Biol. *21*, 47–50.
2. Voeltz, G.K., and Prinz, W.A. (2007). Sheets, ribbons and tubules - how organelles get their shape. Nat. Rev. Mol. Cell Biol. *8*, 258–264.
3. Zimmerberg, J., and Kozlov, M.M. (2006). How proteins produce cellular membrane curvature. Nat. Rev. Mol. Cell Biol. *7*, 9–19.
4. Schmid, S.L. (1993). Biochemical requirements for the formation of clathrin- and COP-coated transport vesicles. Curr. Opin. Cell Biol. *5*, 621–627.
5. Kaksonen, M., Toret, C.P., and Drubin, D.G. (2005). A modular design for the clathrin- and actin-mediated endocytosis machinery. Cell *123*, 305–320.
6. McMahon, H.T., and Gallop, J.L. (2005). Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature *438*, 590–596.
7. Misteli, T. (2001). The concept of self-organization in cellular architecture. J. Cell Biol. *155*, 181–185.
8. Lippincott-Schwartz, J., Cole, N.B., and Donaldson, J.G. (1998). Building a secretory apparatus: role of ARF1/COPI in Golgi biogenesis and maintenance. Histochem. Cell Biol. *109*, 449–462.
9. Dubin-Thaler, B.J., Hofman, J.M., Cai, Y., Xenias, H., Spielman, I., Shneidman, A.V., David, L.A., Dobereiner, H.G., Wiggins, C.H., and Sheetz, M.P. (2008). Quantification of cell edge velocities and traction forces reveals distinct motility modules during cell spreading. PLoS ONE *3*, e3735.
10. Maeda, Y.T., Inose, J., Matsuo, M.Y., Iwaya, S., and Sano, M. (2008). Ordered patterns of cell shape and orientational correlation during spontaneous cell migration. PLoS ONE *3*, e3734.
11. Engelman, D.M. (2005). Membranes are more mosaic than fluid. Nature *438*, 578–580.
12. Singer, S.J., and Nicolson, G.L. (1972). The fluid mosaic model of the structure of cell membranes. Science *175*, 720–731.
13. Loerke, D., Mettlen, M., Yarar, D., Jaqaman, K., Jaqaman, H., Danuser, G., and Schmid, S.L. (2009). Cargo and dynamin regulate clathrin-coated pit maturation. PLoS Biol. *7*, e57.
14. Bannykh, S.I., Plutner, H., Matteson, J., and Balch, W.E. (2005). The role of ARF1 and rab GTPases in polarization of the Golgi stack. Traffic *6*, 803–819.
15. Rappoport, J.Z., Simon, S.M., and Benmerah, A. (2004). Understanding living clathrin-coated pits. Traffic *5*, 327–337.
16. Veksler, A., and Gov, N.S. (2007). Phase transitions of the coupled membrane-cytoskeleton modify cellular shape. Biophys. J. *93*, 3798–3810.
17. Doherty, G.J., and McMahon, H.T. (2008). Mediation, modulation, and consequences of membrane-cytoskeleton interactions. Annu. Rev. Biophys. *37*, 65–95.
18. van Meer, G., and Sprong, H. (2004). Membrane lipids and vesicular traffic. Curr. Opin. Cell Biol. *16*, 373–378.
19. Parton, R.G., and Richards, A.A. (2003). Lipid rafts and caveolae as portals for endocytosis: new insights and common mechanisms. Traffic *4*, 724–738.
20. Martin, S., and Parton, R.G. (2005). Caveolin, cholesterol, and lipid bodies. Semin. Cell Dev. Biol. *16*, 163–174.
21. Baba, T., Rauch, C., Xue, M., Terada, N., Fujii, Y., Ueda, H., Takayama, I., Ohno, S., Farge, E., and Sato, S.B. (2001). Clathrin-dependent and clathrin-independent endocytosis are differentially sensitive to insertion of poly (ethylene glycol)-derivatized cholesterol in the plasma membrane. Traffic *2*, 501–512.
22. van Blitterswijk, W.J., van der Luit, A.H., Veldman, R.J., Verheij, M., and Borst, J. (2003). Ceramide: second messenger or modulator of membrane structure and dynamics? Biochem. J. *369*, 199–211.
23. Kanaseki, T., and Kadota, K. (1969). The "vesicle in a basket". A morphological study of the coated vesicle isolated from the nerve endings of the guinea pig brain, with special reference to the mechanism of membrane movements. J. Cell Biol. *42*, 202–220.
24. Stagg, S.M., LaPointe, P., and Balch, W.E. (2007). Structural design of cage and coat scaffolds that direct membrane traffic. Curr. Opin. Struct. Biol. *17*, 221–228.
25. Weidman, P., Roth, R., and Heuser, J. (1993). Golgi membrane dynamics imaged by freeze-etch electron microscopy: views of different membrane coatings involved in tubulation versus vesiculation. Cell *75*, 123–133.
26. Schmid, S.L., and Damke, H. (1995). Coated vesicles: a diversity of form and function. FASEB J. *9*, 1445–1453.
27. Nossal, R. (2001). Energetics of clathrin basket assembly. Traffic *2*, 138–147.
28. Takano, K., Toyooka, K., and Suetsugu, S. (2008). EFC/F-BAR proteins and the N-WASP-WIP complex induce membrane curvature-dependent actin polymerization. EMBO J. *27*, 2817–2828.
29. Gallop, J.L., Jao, C.C., Kent, H.M., Butler, P.J., Evans, P.R., Langen, R., and McMahon, H.T. (2006). Mechanism of endophilin N-BAR domain-mediated membrane curvature. EMBO J. *25*, 2898–2910.
30. Ford, M.G., Mills, I.G., Peter, B.J., Vallis, Y., Praefcke, G.J., Evans, P.R., and McMahon, H.T. (2002). Curvature of clathrin-coated pits driven by epsin. Nature *419*, 361–366.
31. Hinrichsen, L., Meyerholz, A., Groos, S., and Ungewickell, E.J. (2006). Bending a membrane: how clathrin affects budding. Proc. Natl. Acad. Sci. USA *103*, 8715–8720.
32. Zimmerberg, J., and McLaughlin, S. (2004). Membrane curvature: how BAR domains bend bilayers. Curr. Biol. *14*, R250–R252.
33. Legendre-Guillemin, V., Wasiak, S., Hussain, N.K., Angers, A., and McPherson, P.S. (2004). ENTH/ANTH proteins and clathrin-mediated membrane budding. J. Cell Sci. *117*, 9–18.
34. McMahon, H.T., and Mills, I.G. (2004). COP and clathrin-coated vesicle budding: different pathways, common approaches. Curr. Opin. Cell Biol. *16*, 379–391.
35. Zhang, F., Yim, Y.I., Scarselletta, S., Norton, M., Eisenberg, E., and Greene, L.E. (2007). Clathrin adaptor GGA1 polymerizes clathrin into tubules. J. Biol. Chem. *282*, 13282–13289.
36. Meyerholz, A., Hinrichsen, L., Groos, S., Esk, P.C., Brandes, G., and Ungewickell, E.J. (2005). Effect of clathrin assembly lymphoid myeloid leukemia protein depletion on clathrin coat formation. Traffic *6*, 1225–1234.
37. Ungewickell, E.J., and Hinrichsen, L. (2007). Endocytosis: clathrin-mediated membrane budding. Curr. Opin. Cell Biol. *19*, 417–425.
38. Rappoport, J.Z., Kemal, S., Benmerah, A., and Simon, S.M. (2006). Dynamics of clathrin and adaptor proteins during endocytosis. Am. J. Physiol. Cell Physiol. *291*, C1072–C1081.
39. Frost, A., Perera, R., Roux, A., Spasov, K., Destaing, O., Egelman, E.H., De Camilli, P., and Unger, V.M. (2008). Structural basis of membrane invagination by F-BAR domains. Cell *132*, 807–817.
40. Mattila, P.K., Pykalainen, A., Saarikangas, J., Paavilainen, V.O., Vihinen, H., Jokitalo, E., and Lappalainen, P. (2007). Missing-in-metastasis and IRSp53 deform PI(4,5)P2-rich membranes by an inverse BAR domain-like mechanism. J. Cell Biol. *176*, 953–964.
41. Lipowsky, R. (1992). Budding of membranes induced by intramembrane domains. J. Phys. II France *2*, 1825–1840.
42. Shnyrova, A., Frolov, V.A., and Zimmerberg, J. (2008). ER biogenesis: self-assembly of tubular topology by protein hairpins. Curr. Biol. *18*, R474–R476.
43. Tagawa, A., Mezzacasa, A., Hayer, A., Longatti, A., Pelkmans, L., and Helenius, A. (2005). Assembly and trafficking of caveolar domains in the cell: caveolae as stable, cargo-triggered, vesicular transporters. J. Cell Biol. *170*, 769–779.
44. Nickel, W., Brugger, B., and Wieland, F.T. (1998). Protein and lipid sorting between the endoplasmic reticulum and the Golgi complex. Semin. Cell Dev. Biol. *9*, 493–501.
45. Simons, K., and Vaz, W.L. (2004). Model systems, lipid rafts, and cell membranes. Annu. Rev. Biophys. Biomol. Struct. *33*, 269–295.
46. Simons, K., and Ikonen, E. (1997). Functional rafts in cell membranes. Nature *387*, 569–572.
47. Asp, L., Kartberg, F., Fernandez-Rodriguez, J., Smedh, M., Elsner, M., Laporte, F., Barcena, M., Jansen, K.A., Valentijn, J.A., Koster, A.J., *et al.* (2009). Early stages of Golgi vesicle and tubule formation require diacylglycerol. Mol. Biol. Cell *20*, 780–790.
48. Subtil, A., Gaidarov, I., Kobylarz, K., Lampson, M.A., Keen, J.H., and McGraw, T.E. (1999). Acute cholesterol depletion inhibits clathrin-coated pit budding. Proc. Natl. Acad. Sci. USA *96*, 6775–6780.
49. Khalifat, N., Puff, N., Bonneau, S., Fournier, J.B., and Angelova, M.I. (2008). Membrane deformation under local pH gradient: mimicking mitochondrial cristae dynamics. Biophys. J. *95*, 4924–4933.
50. Fournier, J.B., Khalifat, N., Puff, N., and Angelova, M.I. (2009). Chemically triggered ejection of membrane tubules controlled by intermonolayer friction. Phys. Rev. Lett. *102*, 018102.
51. Lajoie, P., Goetz, J.G., Dennis, J.W., and Nabi, I.R. (2009). Lattices, rafts, and scaffolds: domain regulation of receptor signaling at the plasma membrane. J. Cell Biol. *185*, 381–385.
52. Schmid, E.M., and McMahon, H.T. (2007). Integrating molecular and network biology to decode endocytosis. Nature *448*, 883–888.
53. Di Paolo, G., and De Camilli, P. (2006). Phosphoinositides in cell regulation and membrane dynamics. Nature *443*, 651–657.

54. Vogel, V., and Sheetz, M. (2006). Local force and geometry sensing regulate cell functions. Nat. Rev. Mol. Cell Biol. *7*, 265–275.

55. Bannykh, S.I., and Balch, W.E. (1997). Membrane dynamics at the endoplasmic reticulum-Golgi interface. J. Cell Biol. *138*, 1–4.

56. Gov, N.S., and Gopinathan, A. (2006). Dynamics of membranes driven by actin polymerization. Biophys. J. *90*, 454–469.

57. Hu, J., Shibata, Y., Voss, C., Shemesh, T., Li, Z., Coughlin, M., Kozlov, M.M., Rapoport, T.A., and Prinz, W.A. (2008). Membrane proteins of the endoplasmic reticulum induce high-curvature tubules. Science *319*, 1247–1250.

58. Eisenberg, E., and Greene, L.E. (2007). Multiple roles of auxilin and hsc70 in clathrin-mediated endocytosis. Traffic *8*, 640–646.

59. Chernomordik, L.V., and Kozlov, M.M. (2003). Protein-lipid interplay in fusion and fission of biological membranes. Annu. Rev. Biochem. *72*, 175–207.

60. Chernomordik, L.V., Leikina, E., Kozlov, M.M., Frolov, V.A., and Zimmerberg, J. (1999). Structural intermediates in influenza haemagglutinin-mediated fusion. Mol. Membr. Biol. *16*, 33–42.

61. Graham, T.R. (2004). Flippases and vesicle-mediated protein transport. Trends Cell Biol. *14*, 670–677.

62. Campelo, F., McMahon, H.T., and Kozlov, M.M. (2008). The hydrophobic insertion mechanism of membrane curvature generation by proteins. Biophys. J. *95*, 2325–2339.

63. Reynwar, B.J., Illya, G., Harmandaris, V.A., Muller, M.M., Kremer, K., and Deserno, M. (2007). Aggregation and vesiculation of membrane proteins by curvature-mediated interactions. Nature *447*, 461–464.

64. Louvet-Vallee, S. (2000). ERM proteins: from cellular architecture to cell signaling. Biol. Cell *92*, 305–316.

65. Kohyama, T., Kroll, D.M., and Gompper, G. (2003). Budding of crystalline domains in fluid membranes. Phys. Rev. E Stat. Nonlin. Soft. Matter Phys. *68*, 061905.

66. Sens, P., Johannes, L., and Bassereau, P. (2008). Biophysical approaches to protein-induced membrane deformations in trafficking. Curr. Opin. Cell Biol. *20*, 476–482.

67. Sorre, B., Callan-Jones, A., Manneville, J.B., Nassoy, P., Joanny, J.F., Prost, J., Goud, B., and Bassereau, P. (2009). Curvature-driven lipid sorting needs proximity to a demixing point and is aided by proteins. Proc. Natl. Acad. Sci. USA *106*, 5622–5626.

68. Tian, A., and Baumgart, T. (2009). Sorting of lipids and proteins in membrane curvature gradients. Biophys. J. *96*, 2676–2688.

69. Cooke, I.R., and Deserno, M. (2006). Coupling between lipid shape and membrane curvature. Biophys. J. *91*, 487–495.

70. Mouritsen, O.G. (2005). Life-as a Matter of Fat: The Emerging Science of Lipidomics (New York, LLC: Springer-Verlag).

71. Epand, R.M. (2007). Membrane lipid polymorphism: relationship to bilayer properties and protein function. Methods Mol. Biol. *400*, 15–26.

72. Fuller, N., and Rand, R.P. (2001). The influence of lysolipids on the spontaneous curvature and bending elasticity of phospholipid membranes. Biophys. J. *81*, 243–254.

73. Leikin, S., Kozlov, M.M., Fuller, N.L., and Rand, R.P. (1996). Measured effects of diacylglycerol on structural and elastic properties of phospholipid membranes. Biophys. J. *71*, 2623–2632.

74. Lundmark, R., Doherty, G.J., Vallis, Y., Peter, B.J., and McMahon, H.T. (2008). Arf family GTP loading is activated by, and generates, positive membrane curvature. Biochem. J. *414*, 189–194.

75. Bi, X., Corpina, R.A., and Goldberg, J. (2002). Structure of the Sec23/24-Sar1 pre-budding complex of the COPII vesicle coat. Nature *419*, 271–277.

76. Sheetz, M.P., and Singer, S.J. (1974). Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte interactions. Proc. Natl. Acad. Sci. USA *71*, 4457–4461.

77. Frese, R.N., Pamies, J.C., Olsen, J.D., Bahatyrova, S., van der Weij-de Wit, C.D., Aartsma, T.J., Otto, C., Hunter, C.N., Frenkel, D., and van Grondelle, R. (2008). Protein shape and crowding drive domain formation and curvature in biological membranes. Biophys. J. *94*, 640–647.

78. Frolov, V.A., and Zimmerberg, J. (2008). Flexible scaffolding made of rigid BARs. Cell *132*, 727–729.

79. Frost, A., Unger, V.M., and De Camilli, P. (2009). The BAR domain superfamily: membrane-molding macromolecules. Cell *137*, 191–196.

80. Saarikangas, J., Zhao, H., Pykalainen, A., Laurinmaki, P., Mattila, P.K., Kinnunen, P.K., Butcher, S.J., and Lappalainen, P. (2009). Molecular mechanisms of membrane deformation by I-BAR domain proteins. Curr. Biol. *19*, 95–107.

81. Yang, C., Hoelzle, M., Disanza, A., Scita, G., and Svitkina, T. (2009). Coordination of membrane and actin cytoskeleton dynamics during filopodia protrusion. PLoS One *4*, e5678.

82. Limozin, L., Barmann, M., and Sackmann, E. (2003). On the organization of self-assembled actin networks in giant vesicles. Eur. Phys. J. E Soft. Matter *10*, 319–330.

83. Liu, A.P., Richmond, D.L., Maibaum, L., Pronk, S., Geissler, P.L., and Fletcher, D.A. (2008). Membrane-induced bundling of actin filaments. Nat. Phys. *4*, 789–793.

84. Schein, S., and Sands-Kidner, M. (2008). A geometric principle may guide self-assembly of fullerene cages from clathrin triskelia and from carbon atoms. Biophys. J. *94*, 958–976.

85. Kooijman, E.E., Chupin, V., Fuller, N.L., Kozlov, M.M., de Kruijff, B., Burger, K.N., and Rand, P.R. (2005). Spontaneous curvature of phosphatidic acid and lysophosphatidic acid. Biochemistry *44*, 2097–2102.

86. Shemesh, T., Luini, A., Malhotra, V., Burger, K.N., and Kozlov, M.M. (2003). Prefission constriction of Golgi tubular carriers driven by local lipid metabolism: a theoretical model. Biophys. J. *85*, 3813–3827.

87. Koster, G., Cacciuto, A., Derenyi, I., Frenkel, D., and Dogterom, M. (2005). Force barriers for membrane tube formation. Phys. Rev. Lett. *94*, 068101.

88. Manneville, J.B., Bassereau, P., Levy, D., and Prost, J. (1999). Activity of transmembrane proteins induces magnification of shape fluctuations of lipid membranes. Phys. Rev. Lett. *82*, 4356–4359.

89. Girard, P., Prost, J., and Bassereau, P. (2005). Passive or active fluctuations in membranes containing proteins. Phys. Rev. Lett. *94*, 088102.

90. Fletcher, D.A., and Geissler, P.L. (2009). Active biological materials. Annu. Rev. Phys. Chem. *60*, 469–486.

91. Chen, H.Y. (2004). Internal states of active inclusions and the dynamics of an active membrane. Phys. Rev. Lett. *92*, 168101.

92. Heinzer, S., Worz, S., Kalla, C., Rohr, K., and Weiss, M. (2008). A model for the self-organization of exit sites in the endoplasmic reticulum. J. Cell Sci. *121*, 55–64.

93. Karsenti, E. (2008). Self-organization in cell biology: a brief history. Nat. Rev. Mol. Cell Biol. *9*, 255–262.

94. Foret, L., and Sens, P. (2008). Kinetic regulation of coated vesicle secretion. Proc. Natl. Acad. Sci. USA *105*, 14763–14768.

95. Weiss, M., and Nilsson, T. (2003). A kinetic proof-reading mechanism for protein sorting. Traffic *4*, 65–73.

96. Merrifield, C.J., Perrais, D., and Zenisek, D. (2005). Coupling between clathrin-coated-pit invagination, cortactin recruitment, and membrane scission observed in live cells. Cell *121*, 593–606.

97. Romer, W., Berland, L., Chambon, V., Gaus, K., Windschiegl, B., Tenza, D., Aly, M.R., Fraisier, V., Florent, J.C., Perrais, D., *et al*. (2007). Shiga toxin induces tubular membrane invaginations for its uptake into cells. Nature *450*, 670–675.

98. Mettlen, M., Stoeber, M., Loerke, D., Antonescu, C.N., Danuser, G., and Schmid, S.L. (2009). Endocytic accessory proteins are functionally distinguished by their differential effects on the maturation of clathrin-coated pits. Mol. Biol. Cell *20*, 3251–3260.

99. Frick, M., Bright, N.A., Riento, K., Bray, A., Merrified, C., and Nichols, B.J. (2007). Coassembly of flotillins induces formation of membrane microdomains, membrane curvature, and vesicle budding. Curr. Biol. *17*, 1151–1156.

100. Donaldson, J.G. (2008). Arfs and membrane lipids: sensing, generating and responding to membrane curvature. Biochem. J. *414*, e1–e2.

101. Goulian, M., Bruinsma, R., and Pincus, P. (1993). Long-range forces in heterogeneous fluid membranes. Europhys. Lett. *22*, 145–150.

102. Botelho, A.V., Huber, T., Sakmar, T.P., and Brown, M.F. (2006). Curvature and hydrophobic forces drive oligomerization and modulate activity of rhodopsin in membranes. Biophys. J. *91*, 4464–4477.

103. Bagatolli, L.A., and Gratton, E. (1999). Two-photon fluorescence microscopy observation of shape changes at the phase transition in phospholipid giant unilamellar vesicles. Biophys. J. *77*, 2090–2101.

104. Heuser, J., and Kirchhausen, T. (1985). Deep-etch views of clathrin assemblies. J. Ultrastruct. Res. *92*, 1–27.

105. Greene, B., Liu, S.H., Wilde, A., and Brodsky, F.M. (2000). Complete reconstitution of clathrin basket formation with recombinant protein fragments: adaptor control of clathrin self-assembly. Traffic *1*, 69–75.

106. Pollard, T.D., and Borisy, G.G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. Cell *112*, 453–465.

107. Giannone, G., Dubin-Thaler, B.J., Dobereiner, H.G., Kieffer, N., Bresnick, A.R., and Sheetz, M.P. (2004). Periodic lamellipodial contractions correlate with rearward actin waves. Cell *116*, 431–443.

108. Bigay, J., Gounon, P., Robineau, S., and Antonny, B. (2003). Lipid packing sensed by ArfGAP1 couples COPI coat disassembly to membrane bilayer curvature. Nature *426*, 563–566.

109. Lippincott-Schwartz, J., and Liu, W. (2003). Membrane trafficking: coat control by curvature. Nature *426*, 507–508.

110. Aridor, M., Bannykh, S.I., Rowe, T., and Balch, W.E. (1999). Cargo can modulate COPII vesicle formation from the endoplasmic reticulum. J. Biol. Chem. *274*, 4389–4399.

111. Leduc, C., Campas, O., Zeldovich, K.B., Roux, A., Jolimaitre, P., Bourel-Bonnet, L., Goud, B., Joanny, J.F., Bassereau, P., and Prost, J. (2004). Cooperative extraction of membrane nanotubes by molecular motors. Proc. Natl. Acad. Sci. USA *101*, 17096–17101.

112. Watson, P., and Stephens, D.J. (2005). ER-to-Golgi transport: form and formation of vesicular and tubular carriers. Biochim. Biophys. Acta *1744*, 304–315.

113. Kress, H., Stelzer, E.H., Holzer, D., Buss, F., Griffiths, G., and Rohrbach, A. (2007). Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity. Proc. Natl. Acad. Sci. USA *104*, 11633–11638.

114. Heinrich, V., Bozic, B., Svetina, S., and Zeks, B. (1999). Vesicle deformation by an axial load: from elongated shapes to tethered vesicles. Biophys. J. *76*, 2056–2071.

115. Frolov, V.A., Lizunov, V.A., Dunina-Barkovskaya, A.Y., Samsonov, A.V., and Zimmerberg, J. (2003). Shape bistability of a membrane neck: a toggle switch to control vesicle content release. Proc. Natl. Acad. Sci. USA *100*, 8698–8703.

116. Bashkirov, P.V., Akimov, S.A., Evseev, A.I., Schmid, S.L., Zimmerberg, J., and Frolov, V.A. (2008). GTPase cycle of dynamin is coupled to membrane squeeze and release, leading to spontaneous fission. Cell *135*, 1276–1286.

117. Rauch, C., and Farge, E. (2000). Endocytosis switch controlled by transmembrane osmotic pressure and phospholipid number asymmetry. Biophys. J. *78*, 3036–3047.

118. Fourcade, B., Miao, L., Rao, M., Wortis, M., and Zia, R.K. (1994). Scaling analysis of narrow necks in curvature models of fluid lipid-bilayer vesicles. Phys. Rev. E Stat. Phys. Plasmas Fluids Relat. Interdiscip. Topics *49*, 5276–5286.

119. Sieber, J.J., Willig, K.I., Kutzner, C., Gerding-Reimers, C., Harke, B., Donnert, G., Rammner, B., Eggeling, C., Hell, S.W., Grubmuller, H., *et al*. (2007). Anatomy and dynamics of a supramolecular membrane protein cluster. Science *317*, 1072–1076.

120. Welsch, S., Muller, B., and Krausslich, H.G. (2007). More than one door - Budding of enveloped viruses through cellular membranes. FEBS Lett. *581*, 2089–2097.

121. Hurley, J.H., and Emr, S.D. (2006). The ESCRT complexes: structure and mechanism of a membrane-trafficking network. Annu. Rev. Biophys. Biomol. Struct. *35*, 277–298.

122. Pucadyil, T.J., and Schmid, S.L. (2008). Real-time visualization of dynamin-catalyzed membrane fission and vesicle release. Cell *135*, 1263–1275.